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Denitrification in Low pH Spodosols and Peats Determined with the Acetylene Inhibition Method

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Potential denitrification rates were determined for predominantly acid (pH ≥ 3.6) horizons of forestal, miry, and agricultural soils from 22 locations in southern Finland. The acetylene inhibition method was used with nitrate-amended waterlogged soils incubated in an N_2 atmosphere containing 2.5 or 5% C_2H_2. Complete inhibition of the reduction of N_2O to N_2 was observed in 99.3% of the samples. The denitrification rates varied from 0.12 to 53.8 μg of $N \cdot cm^{-3} \cdot day^{-1}$. Correlation between denitrification rate and soil pH was highly significant: $r = 0.84$ on a volume basis, and $r = 0.44$ on a weight basis. Vegetation type and amount of soil organic matter had a minor or no effect, respectively. In spodosolized soils the rates were significantly higher for B horizons than for A horizons. These results show that denitrification can occur in acid soils.

Denitrification is strongly affected by many soil variables, including pO_2, carbon availability, and temperature. Furthermore, the denitrifying bacteria are sensitive to low pH (4, 19, 23). It has also been demonstrated that the denitrifying ability of soil is depressed by low pH (6, 12, 14). A complicating factor in such studies may be nonbiological denitrification, since gaseous loss of nitrogen by nonbiological mechanisms may increase with decreasing pH of soil (3, 16). However, studies on denitrification in low pH soils are scarce.

Low pH peats and spodosols are prevalent in Finland. The purpose of this study was to determine, without addition of energy sources, the potential denitrification rates of these soils. The denitrification rates were determined with the acetylene (C_2H_2) inhibition method. Since Balderston et al. (2) and Yoshinari and Knowles (27) reported that acetylene inhibited the reduction of N_2O to N_2 in pure cultures of denitrifying organisms, a number of investigators (12, 17, 20, 26) have successfully used the C_2H_2 inhibition method to assay denitrification in soils.

MATERIALS AND METHODS

Soils. Characteristics of soils from 22 locations in southern Finland are listed in Table 1. Within the major groups (mire, forest, etc.) the soils are listed roughly in order of trophic state. Soil analysis and biological characteristics will be described in full elsewhere (Niemi et al., submitted for publication). With the exception of SK-1, SK-2, and KK-8, all sampling sites were located in non-agricultural areas; SK areas were ploughed to a depth of 35 to 45 cm before sampling. Four to five subsamples (500 cm^3) of each soil horizon were taken from a 10-m^2 area (three subsamples were taken from areas KK-7, SK-1, and SK-2). Aerobic and anaerobic peat layer samples were distinguished by the smell of H_2S. All samples were collected in August and September 1978 and transported to the laboratory in plastic bags. Samples from the same sampling area and the same horizon were pooled. Part of the soil was air dried and stored at 22°C for chemical analyses, and the rest was stored at −18°C until it was required for the denitrification rate assays. The soil pH values were determined in a soil-water suspension (2:1 [vol/vol]). The carbon content was determined with an infrared carbon analyzer (URAS-2T) by high-temperature combustion (18). Combined NO_2^- and NO_3^- was determined from 2 N KCl extracts by alkaline steam distillation (5).

Incubation. Five replicates of each soil sample (12 cm^3 of fresh soil, except for SK-1 soil, the samples of which corresponded to 5 g of oven-dry weight) were incubated in 20 ml of water (25 ml for SK-1) in 210-ml glass flasks closed with rubber septum stoppers. The amount of water was sufficient to waterlog the soils. The flasks were evacuated and refilled with N_2 to ambient pressure four times, after which the final pO_2 was < 0.01. An amount of 10 cm^3 (5 cm^3 for SK soils) of acetylene was added to each flask, resulting in a pC_2H_2 of 0.05 (0.025 for SK soils). The soils were preincubated at 18 to 22°C, and the N_2O concentrations in the flask atmospheres were assayed daily. Preincubation was continued for at least 3 days or until formation of N_2O from endogenous nitrate was no longer observed (in some cases not until 5 days), at which time the evacuation procedure and the acetylene addition were repeated. The reason for preincubation was to remove any NO_3^- initially available in the samples, and thus to allow for checking of the stoichiometry of reduction of the added NO_3^-. The incubation was initiated by injecting a solution of KNO_3^- (42 μg of $N \cdot cm^{-3}$ of soil, except SK-2 for which 29 μg of $N \cdot cm^{-3}$ was used) into three of the five flasks (test flasks) and gaseous N_2O (equivalent to

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TABLE 1. *Denitrification potential rates of some Finnish soils*[a]

Soil[b]	Vegetation type[c]	Horizon	Sampling depth (cm)	Soil characteristics					Denitrification rate (μg of N·cm^{-3}·day^{-1})[e]
				Soil type[d]	Organic carbon (% [wt/wt])	Density (g·cm^{-3})	pH	$NO_3^- + NO_2^-$ (μg·cm^{-3})	
Mire									
PY-3	*Sphagnum fuscum* bog	O_1	0–10	LSt-Ae	22.8	0.09	3.6	1.1	2.9 ± 4.4
		O_2	10–40	LSt-An	54.5	0.15	3.7	0.8	2.0 ± 0.4
PY-2	*Eriophorus vaginatum* pine bog	O_1	0–10	St-Ae	58.2	0.06	3.8	1.1	0.63 ± 0.30
		O_2	10–40	ErSt-An	67.3	0.12	3.8	<0.5	2.2 ± 0.4
PY-5	Tall-sedge fen	O_1	0–10	ErSCt-Ae	62.0	0.07	3.9	<0.5	2.8 ± 3.5
		O_2	10–40	SCt-An	61.4	0.11	4.0	<0.5	3.4 ± 0.9
LE-3	Paludified *V. myrtillus* spruce marsh	O	0–15	Kh	50.6	0.13	3.8	<0.5	1.5 ± 1.7
		A	15–25	SrHHk-1	0.9	1.0	3.8	<0.5	0.37 ± 0.07
		B	25–40	SrHHk-2	1.5	1.0	4.8	<0.5	2.7 ± 2.8
JS-1	*Rubus chamaemorus* spruce swamp	O_1	0–10	LSt-Ae	ND[f]	0.08	5.6	ND	23.6 ± 7.6
		O_2	10–30	LSt-An	41.3	0.07	5.8	0.6	28.5 ± 2.7
JS-2	Sedge pine swamp	O_1	0–5	LSCt-Ae	43.6	0.07	5.8	<0.5	37.3 ± 1.9
		O_2	5–30	LSCt-An	58.6	0.06	5.5	<0.5	19.2 ± 10.7
LB-3	*Alnus glutinosa* swamp	O_1	0–10	LCt-Ae	51.7	0.17	5.6	2.9	35.8 ± 2.4
		O_2	10–30	LCt-An	43.2	0.15	5.6	<0.5	13.6 ± 1.4
KK-6	*Alnus glutinosa* swamp	O	0–10	LjHHt	11.7	0.49	5.6	10.3	30.6 ± 1.2
		A	10–30	SrHHk	0.3	1.3	5.9	2.6	3.8 ± 0.8
Forest									
PA-1[g]	*Calluna* pine forest	O	3–5	HkKh	23.8	0.23	3.6	0.9	0.16 ± 0.08
		A	5–7	HHk	0.5	1.2	4.0	1.2	0.35 ± 0.04
		B	7–30	HHk	1.4	1.1	5.0	<0.5	17.8 ± 2.8
LE-1[g]	*Calluna* spruce forest	O	0–5	Kh	29.4	0.21	3.8	<0.5	3.8 ± 5.5
		B	5–30	HHk	0.8	1.1	4.9	<0.5	8.6 ± 2.0
PA-2[g]	*Vaccinium* pine forest	O	0–3	Kh	36.2	0.14	3.6	<0.5	0.12 ± 0.07
		A	3–5	SrHHk	0.8	1.1	3.9	<0.5	0.58 ± 0.65
		B	5–30	HHk	2.8	1.1	4.6	<0.5	14.4 ± 10.4
LE-2[g]	*Vaccinium* spruce forest	O	0–5	Kh	37.2	0.23	4.3	<0.5	1.6 ± 0.2
		B	5–30	HHk	0.9	1.5	5.1	<0.5	14.5 ± 4.7
PA-3[g]	*Myrtillus* spruce forest	O	0–10	Kh	49.2	0.23	3.6	<0.5	0.92 ± 0.59
		A	10–15	KHk	1.3	1.0	3.8	<0.5	1.5 ± 0.2
		B	15–30	HkKHk	2.8	0.88	4.5	1.4	6.3 ± 1.0
PY-1	*Oxalis-Myrtillus* spruce forest	O	0–5	HHt	10.2	0.37	5.0	8.5	30.2 ± 7.8
		A	5–40	HHt	2.1	0.81	5.1	<0.5	16.4 ± 0.9
LB-1	*Oxalis-Myrtillus* spruce forest	O	0–5	Lm	17.5	0.33	5.2	<0.5	21.1 ± 1.2
		A	5–30	mKh	6.9	0.62	4.6	0.6	13.8 ± 3.1
KK-4	*Melica-Lathyrus* deciduous forest	O	0–10	Lm(Hk)	8.6	0.38	5.3	<0.5	33.9 ± 13.7
		A	10–20	HtMr	4.1	0.74	5.4	0.7	25.7 ±13.5
LB-2	*Oxalis-Maianthemum* deciduous forest	O	0–20	KHt	6.2	0.70	4.7	3.5	17.1 ± 1.8
KK-5	*Oxalis-Maianthemum* deciduous forest	O	0–5	LjSHHt	5.2	0.59	5.7	1.2	45.6 ± 11.0
		A	10–20	HtS	4.4	0.71	5.5	<0.5	19.0 ± 2.7
Meadow and agricultural									
KK-7	Natural meadow	O	0–5	Lm	11.6	0.35	6.6	0.7	32.0 ± 1.9
		A	5–25	Lm	11.6	0.53	7.3	2.1	53.8 ± 8.0
KK-8	Previously cultivated meadow	L	0–5	Litter	7.7	0.54	5.9	1.1	37.1 ± 3.7
		Cultivated	5–15	rmKHt	3.1	0.9	5.9	<0.5	21.9 ± 0.3
		A	15–30	HtMr	0.7	1.2	5.9	<0.5	13.5 ± 4.0
SK-1	Cultivated	Cultivated	0–20	Lm	43.9	0.15	5.3	3.3	21.1 ± 0.7
SK-2	Cultivated	Cultivated	0–40	HHs	1.5	0.73	5.8	3.7	10.1 ± 5.0

[a] Soils are listed in increasing trophic order of sampling site. All weights refer to the oven-dry weight, and all volumes refer to fresh soil volumes.

[b] Letters refer to location: JS, Janakkala-Suurisuo; KK, Karjalohja-Karkali; LB, Lammi-Biological station; LE, Lammi-Evo; PA, Parkano-Alkkianvuori; PY, Parkano-Ylimysneva; SK, Suomusjärvi-Kettula.

[c] Forest according to Cajander (8) and mires according to Pakarinen and Ruuhijärvi (15).

[d] Soil type codes (1): Ae, aerobic peat layer; An; anaerobic peat layer; ErSCt, *Eriophorum-Sphagnum-Carex* peat; ErSt, *Eriophorum-Sphagnum* peat; HHk, finer sand; HHs, fine silt; HHt, finer finesand; HkKh, sandy mor humus; HkKHk, sandy coarse sand; HtMr, finesand moraine; HtS, sandy clay; Kh, mor humus; KHk, coarse sand; KHt, finesand; LCt, ligno-*Carex* peat; LjHHt, finer finesand gyttja; LjSHHt, clay-finesand gyttja; Lm, mould humus; Lm(Hk), sandy mould humus; LSCt, ligno-*Sphagnum-Carex* peat; LSt, ligno-*Sphagnum* peat; mKh, mouldy mor humus; rmKHt, mouldy finesand; SCt, *Sphagnum-Carex* peat; SrHHk, finer sandy gravel; St, *Sphagnum* peat.

[e] Averages of triplicates ± standard deviation.

[f] ND, Not determined.

[g] Spodosolized soil. LE soils were less spodosolized than PA soils.

NO_3^- nitrogen added to test flasks) into the remaining two flasks (control flasks). After addition of NO_3^-, the samples were incubated at 18 to 22°C for 6 to 21 days. In a separate experiment, KNO_3, varying from 6 to 180 μg of $N \cdot cm^{-3}$, was added to SK-1 soil. The pressure in the flasks did not increase measurably during incubation. The soil pH increased by 0 to 0.75 units.

Assay of N_2O. Gas samples (0.5 cm^3) were withdrawn from the flasks with a gastight syringe, and analyzed for N_2O (and O_2) by gas chromatography (Perkin-Elmer F11 equipped with a ^{63}Ni electron capture detector and a stainless-steel Porapak Q 80/100 mesh column 2 m in length with a 2-mm inner diameter). Temperatures in the injector port, oven, and detector were 130, 50, and 140°C, respectively. The carrier gas (N_2) flow was 30 cm^3/min. The calculation of the amount of N_2O formed in the test flasks was based on a standard curve of N_2O dilution and corrected for N_2O solubility in the water-soil suspension by using averaged N_2O solubility values obtained from the gas phase of the two control flasks for each soil. It was determined that 11 ± 3% of the total N_2O was dissolved in the soil-water suspension. The added N_2O equilibrated between the liquid and gas phases within 2 h. N_2O was determined at fixed intervals after addition of NO_3^- (see Fig. 2). The potential denitrification rates given were estimated from the two successive N_2O measurements which gave the highest rate.

RESULTS

The NO_3^- added to SK-1 soil was recovered stoichiometrically as N_2O after incubation periods which increased in proportion to the amount of NO_3^- added (Fig. 1). The N_2O production patterns varied among the soils examined. Examples of different patterns are presented in Fig. 2. The KK-5 O horizon had one of the highest denitrifying activities and showed the maximum rate of N_2O production 15 to 25 h after addition of nitrate. In the case of the LE-2 B horizon, the maximum rate was not reached until 3 days after addition of nitrate. The LB-3 anaerobic peat produced N_2O at a constant rate during a period of 78 h. After a lag period, these soils denitrified stoichiometrically the total added NO_3^- to N_2O. The PY-2 aerobic peat represented a slowly denitrifying soil. In no case did the N_2O production cease before all the added NO_3^- was converted to N_2O.

In 53% of the samples, the added NO_3^- was

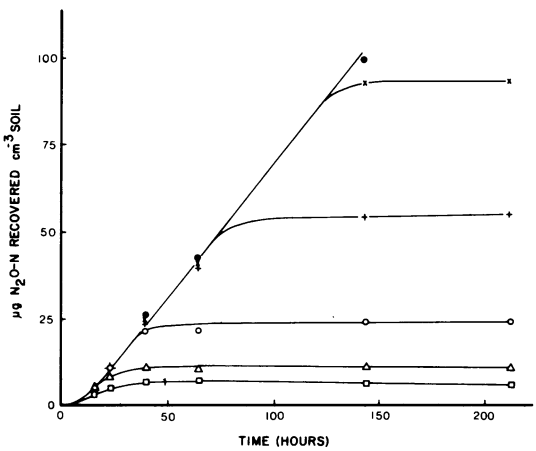

FIG. 1. *Production of N_2O by SK-1 soil amended with KNO_3 at concentrations of 6 (□), 12 (△), 24 (○), 54 (+), 90 (×), and 180 (●) μg of $N \cdot cm^{-3}$. The soil was incubated in water in an N_2 atmosphere, pC_2H_2 = 0.025, at 18 to 22°C. The results shown are averages of two replicates.*

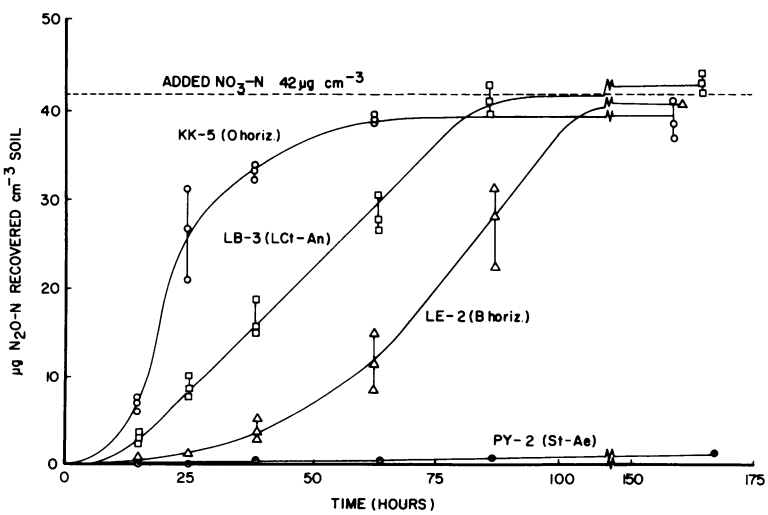

FIG. 2. *Four examples of different N_2O production patterns observed. The soils were amended with NO_3^- (42 μg of $N \cdot cm^{-3}$), incubated in water in an N_2 atmosphere, pC_2H_2 = 0.05, at 18 to 22°C. The results of each replicate are shown.*

reduced stoichiometrically to N_2O during incubation. In the remaining 47%, incubation was terminated before completion of the NO_3^- reduction, i.e., while the N_2O production still continued. In soils of pH < 4.5, only 3 to 10% of the added NO_3^- was reduced to N_2O during incubation. A decrease in N_2O was observed in 2 of the 286 flasks analyzed. This was not caused by leakage of gas, because acetylene and oxygen concentrations did not change during incubation.

The denitrification rates of the soils, measured as N_2O (Table 1), varied between 0.12 and 53.8 μg of $N \cdot cm^{-3} \cdot day^{-1}$, and their correlation with soil pH values was highly significant; $r = 0.84$, significant at $P < 0.001$, for denitrification expressed per volume of soil; and $r = 0.44$, significant at $P < 0.01$, an oven-dry weight basis (Fig. 3). No significant correlation was observed between denitrification rates and other soil characteristics such as organic carbon ($r = -0.19$), total nitrogen, combined NO_3^- and NO_2^-, exchangeable ammonia content, and fixed ammonia content (Niemi et al., submitted for publication).

The denitrification rates of spodosolized soils were considerably higher in B horizons than in O and A horizons, reflecting the higher pH values of the B horizons. The average denitrification rate was 1.1 μg of $N \cdot cm^{-3} \cdot day^{-1}$ for the O and A horizons and 12.3 μg of $N \cdot cm^{-3} \cdot day^{-1}$ for the B horizons (sites PA-1, PA-2, PA-3, LE-1, and LE-2). Differences between these potential rates were less pronounced in the LE soils than in the PA soils, the former being less spodosolized.

DISCUSSION

Inhibition of denitrification by acetylene was almost complete in our experimental conditions. Yeomans and Beauchamp (25) reported for Huron silty clay loam a rapid reduction of N_2O after 5 to 7 days of incubation in a 1% atmosphere of C_2H_2. In contrast, we observed in only 2 of the 286 samples analyzed a decrease of N_2O during 6 to 21 days of incubation in a 5% atmosphere of C_2H_2. The stoichiometrical reduction of added NO_3^- to N_2O, observed in all the samples in which N_2O production had ceased, shows that no NO_3^- was assimilated and also that the NO, possibly produced in the beginning, had been further reduced to N_2O in these samples. Additionally, no NO_3^- was reduced to NH_4^+, which is consistent with some earlier investigations (9, 14, 24) in which very small amounts of NH_4^+ have been observed to be formed in unamended soils. In soils of pH < 4.5, only 3 to 10% of the added NO_3^- was reduced to N_2O during incubation. Because low pH in soil enhances production of NO and NO_2 (3, 16, 24), it is possible that NO and NO_2 in these samples were products in addition to N_2O. Consequently, the denitrification rates given for the most acid soils may be underestimations. On the other hand, studies with soils of pH ≥ 5.8 and ^{13}N or ^{15}N methodologies have shown that the rate and extent of denitrification in the presence of C_2H_2 are nearly equal or equal, respectively, to those found in the absence of C_2H_2 (17, 20).

Several workers (7, 11, 22) have ascertained no correlation between the denitrification rate and pH of soils. The number of low pH soils was limited in those investigations. Some other workers (6, 10) have found a correlation, and a significant correlation became apparent also in our work, which included many soils of low pH. Irrespective of the widely differing types of soil and vegetation, denitrification rates in soils of similar pH were of equal magnitude; for example, the JS-2 (sedge-pine swamp) aerobic layer

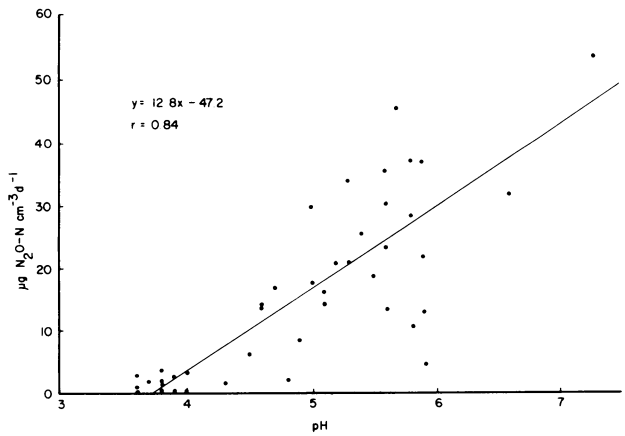

FIG. 3. *Correlation between denitrification rates and soil pH.*

of pH 5.8, the KK-5 (deciduous forest) O horizon of pH 5.7, and the KK-8 (abandoned cultivated meadow) L horizon of pH 5.9 had denitrification rates of 37.3, 45.6, and 37.1 μg of $N \cdot cm^{-3} \cdot day^{-1}$, respectively. On the other hand, denitrification rates for two soils with similar vegetation type, LB-2 and KK-5, differed significantly, but correlated with pH (Table 1).

Many of the soils rich in organic carbon, such as peats and O horizons of spodosols, had a low pH, which may partly explain the lack of correlation between the potential denitrification rates and the organic carbon content. In addition, the available carbon obviously does not correlate with total organic carbon (7, 22).

Due to preincubation, our results probably represent the soil denitrification phases IIa (constant rate) or IIb (logarithmically increasing rate) described by Smith and Tiedje (21). Effects caused by the disturbance of the natural soil structure are difficult to assess, and freezing of the soil may have caused higher denitrification activity than would have been observed from corresponding fresh soil (13).

The denitrification rates observed indicate that denitrification is possible in low pH spodosolized soils and in peats. Although the rates for the most acid soils are considerably lower than those for soils of pH > 5, it must be noted that a denitrification rate of as little as 2 μg of $N \cdot cm^{-3} \cdot day^{-1}$ is equal to a rate of 180 kg of $N \cdot ha^{-1} \cdot month^{-1}$ (calculated for a layer 30 cm thick). However, the denitrification rates we found are certainly upper limits for denitrification in situ, because the conditions in vitro—supply of NO_3^- and control of temperature, anaerobiosis, and moisture—favor denitrification.

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